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PCT/EP03/13655

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29518P WO/WWTGkt

DATUM / DATE

1. - 8. April 2004 *Ant*

Application/Patent No:

PCT/EP03/13655

Applicant/Proprietor:

DeveloGen Aktiengesellschaft für entwicklungsbiologische
Forschung

Encl

Computer disc containing
sequence ✓ listing; paper
copy; ✓ amended description
page 52. ✓

This is in response to the official
communication dated 11.03.2004.

Applicant hereby submits a sequence listing
in computer-readable form according to
WIPO Standard St.25, as well as in the form
of a paper copy.

It is confirmed that the sequence information
recorded on the data carrier is identical to the
written sequence listing. It is further stated
that the sequence listing does not include
subject matter which goes beyond the content
of the application as originally filed.

Applicant also submits herewith amended

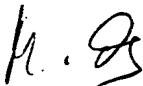
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- 8. April 2004

description page 52 wherein the obvious error in the incorrect sequence listing reference in line 15 „SEQ ID NO. 14“ has been corrected to read „SEQ ID NO. 26“.



Dr M Dey

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Taken together the over-expression of Prl-1 showed an effect on metabolism of exogenous free fatty acids and glucose as well as triglyceride storage in all three assays we performed in SGBS cells, making it a potential interesting drug target for the treatment of diabetes and related metabolic disorders.

5

Example 6: Assays for the determination of lipid storage, synthesis and transport of Prl-1 LOF adipocytes (Figure 6)

Loss of function in 3T3-L1 adipocytes by RNAi technique

10 In order to stably inhibit Prl-1 expression, 3T3-L1 preadipocytes were engineered by retroviral infection aimed to express a target specific short interfering RNA construct under the control of the human hH1 promoter according to Brummelkamp et al. (Science 2002, Vol 296, p. 550-553). The following Prl-1 specific RNAi sequence was used: AGG ATT CCA ATG GTC
15 ATA G (SEQ ID NO. 26).

Retroviral infection of preadipocytes

Packaging cells were transfected with a retroviral plasmid pLPCX carrying the specific RNAi construct under the control of the human hH1 promoter and a
20 selection marker using calcium phosphate procedure. Control cells were infected with the same vector carrying no transgene.

Briefly, exponentially growing packaging cells were seeded at a density of 350,000 cells per 6-well in 2 ml DMEM + 10 % FCS one day before
25 transfection. 10 min before transfection chloroquine was added directly to the overlying medium (25 μ M final concentration). A 250 μ l transfection mix consisting of 5 μ g plasmid-DNA (candidate:helper-virus in a 1:1 ratio) and 250 mM CaCl_2 was prepared in a 15 ml plastic tube. The same volume of 2 x HBS (280 μ M NaCl, 50 μ M HEPES, 1.5 mM Na_2HPO_4 , pH 7.06) was added and air
30 bubbles were injected into the mixture for 15 sec. The transfection mix was added drop wise to the packaging cells, distributed and the cells were incubated at 37°C, 5 % CO_2 for 6 hours. The cells were washed with PBS and the medium was exchanged with 2 ml DMEM + 10 % CS per 6-well. One day after transfection the cells were washed again and incubated for 2 days of
35 virus collection in 1 ml DMEM + 10 % CS per 6-well at 32°C, 5 % CO_2 . The supernatant was then filtered through a 0.45 μ m cellulose acetate filter and